Environmental Health Perspectives
Vol. 79, pp. 243-246, 1989

Quantitative Risk Analysis for Quantal Reproductive and Developmental Effects

by David W. Gaylor*

Animal experiments are generally conducted at higher dose levels than anticipated human dose levels in order to elicit otherwise subtle changes in reproduction or developmental effects with relatively few animals. Based on animal data, regulatory strategy generally has been to postulate a no-observed-effect level (NOEL) for toxic effects and to divide this by a safety factor, usually 100, to establish acceptable levels for humans. Various authors have discussed the shortcomings of using NOEL and have suggested the use of an estimable effect level determined from a dose-response curve fitted to bioassay data, e.g., the dose at which 1% of the animals are adversely affected, and employing some form of conservative low dose extrapolation to control risks at lower doses. In this paper, 10 sets of bioassay data on fetal mortality or anomalies were used to compare the estimated upper limits of risk estimated at the NOEL/100 and the lower 95% confidence limit estimate of the dose producing adverse effects in 1% of the embryonic implants or fetuses divided by 100 ($LED_{01}/100$). The latter quantity is expected to result in a risk (proportion affected) of less than 10^{-4} (1 in 10,000). The estimated upper limits of risk associated with the NOEL/100 were from 2×10^{-4} to 6×10^{-4} for the 10 data sets investigated.

Introduction

In order to detect potential toxic effects with a limited number of animals, experimental studies typically employ doses that are higher than expected human exposure levels. Thus, high to low dose extrapolation is generally required. The following discussion of extrapolation of developmental toxicity data does not discount the possibility of a biological threshold below which no risk exists.

One approach for setting acceptable levels for developmental toxicity risk has been the use of safety (uncertainty) factors. From a bioassay conducted at several dose levels (generally three or more), a supposedly safe dose for humans is determined by dividing the no-observed-effect level (NOEL) by a safety factor. Lehman and Fitzhugh suggested a safety factor of 100 (*1*). If the NOEL is taken to be a safe dose for the experimental animals, a safety factor of 10 is applied to allow for potentially higher sensitivities of humans compared to the experimental animals and another factor of 10 to allow for differences in sensitivities among individuals. For irreversible effects, such as death or malformation, an additional safety factor of 10 is suggested (*2*). Even if a safety factor of 100 is adequate to account for interspecies and intraspecies differences in response, this does not necessarily result in a risk-free dose. The NOEL may not represent a safe dose for the laboratory animal tested because the power of the experiment may be inadequate to detect subtle toxic effects. That is, the NOEL may have been inadequately established. Also, the safety factors are somewhat arbitrary and may be adequate on the average, but may be inadequate for any particular case. Further, Gaylor (*3*) and Crump (*4*) discuss the problem of poorer experiments resulting in higher NOEL's and hence higher allowable levels.

Rather than routinely applying a fixed size safety factor to the NOEL of the critical toxic effect in an animal species to obtain a safe dose, data from the whole dose-response curve should be used in setting acceptable levels for humans that are estimated to be without any appreciable risk. Based upon the upper confidence limit on the risk estimated from the animal dose-response data, the size of the safety factor can be determined to reduce the risk below a certain level for the animal test species (*3*).

Several mathematical dose-response models have been used for curve fitting of experimental data. Crump applied modifications of the one-hit and multistage models to determine a benchmark dose to replace the NOEL (*4*). A benchmark dose was suggested for which the excess risk was estimated not to exceed 1 to 10%, with 95% confidence. Dourson et al. suggested a similar approach (*5*). For carcinogenic data, Mantel and Bryan (*6*), Van Ryzin (*7*), and Farmer et al. (*8*), suggested estimating the dose that produced an excess tumor incidence of 1% (ED_{01}) and then using some

*National Center for Toxicological Research, Jefferson, AR 72079.

form of conservative extrapolation from that point for lower doses. Gaylor suggested using safety factors in conjunction with the ED_{01} for controlling risks at low doses for any toxic effect (*3*).

Regardless of the dose-response model used, it will generally be difficult to estimate with precision an excess risk of less than 1 to 10% above the spontaneous background level for quantal responses, e.g., the proportion of implants that are resorbed or the proportion of live fetuses with a type of malformation, from standard developmental toxicity studies.

Kimmel and Gaylor suggest a procedure based on the ED_{10} for controlling low dose risks (*9*). A dose-response curve is fitted to the experimental data on the proportions affected. This curve is used to estimate the dose that produces a low level of risk in the experimental dose range, e.g., the ED_{10} (the effective dose corresponding to an excess risk of 10%). Then, a lower confidence limit on the dose that produces a 10% risk (the LED_{10}) is obtained. If F represents a safety factor, at a dose of LED_{10}/F, the risk in the low dose region is estimated to be less than $0.1/F$. This procedure is conservative from a safety standpoint when the dose response is curving upward (convex). If a biological threshold exists, the lower limit on risk would be zero if the LED_{10}/F is less than the threshold dose. This procedure makes greater use of the dose-response data collected in animal studies, whereas the current procedure based on the NOEL makes use of only a single point. The advantage of applying safety factors to the LED_{10}, or some other estimable dose, is that an upper limit on the risk can be estimated, whereas application of a safety factor to the NOEL results in variable levels of risk since the potential risk at the NOEL is not considered.

Most teratological studies are capable of detecting disease incidence of 10% or more. Hence, the NOEL will generally represent a dose at which the risk is less than 10%. It generally is possible with a slight extrapolation to estimate the ED_{01}. Since the ED_{01} is generally in the dose range of the NOEL, it is of interest to compare the $LED_{01}/100$ and the NOEL/100. Generally, the ED_{01} will not be directly measurable in a typical teratology study and requires some interpolation or extrapolation from the experimental doses. In general, the choice of the model will not have much impact on the estimation of the ED_{01}. Rather than use the NOEL/100 to set acceptable levels, which can vary considerably depending upon the experimental design, a more consistent approach is to divide the lower confidence limit on the ED_{01} of a given effect, i.e., the LED_{01}, by 100. If the dose response is curving upward in the low-dose region, the risk at the $LED_{01}/100$ is estimated to be less than 10^{-4} (1 in 10,000). If the $LED_{01}/100$ is less than a threshold dose, if one exists, then the risk is zero.

Methods

Ten sets of dose response data for the proportions of fetuses with reproductive or developmental defects were investigated to compare the NOEL/100 and $LED_{01}/100$ procedures (Table 1). In general, it would be preferable to use data on a litter basis. Since the data were not reported for individual litters, dose-response curves on the proportions of dead or abnormal fetuses per litter were not obtained and quantal data on a fetal basis were used.

An exponential polynomial model was fitted to each of the data sets:

$$P = 1 - \exp[-(b_0 + b_1 d + b_2 d^2 + \ldots + b_k d^k)$$

where P was the proportion of affected fetuses, d was the dose rate, and each b was estimated from the data using the procedure of Howe and Crump (*10*). These estimated sigmoidal dose-response curves were only used to provide an estimate of the LED_{01}. Since the estimated dose response is not used for low dose extrapolation, a biologically based model is not particularly important, and all that is required is a descriptive model in the experimental dose range.

Conservative upper limits on low dose risk estimates are obtained for convex (upward curvature) dose-response curves by using a risk to dose slope of $0.01/LED_{01}$, below the LED_{01}, i.e.,

$$\text{Estimated risk} \leq \frac{0.01}{LED_{01}} \times \text{dose}.$$

Thus, the risk at the $LED_{01}/100$ is estimated to be less than 10^{-4} (1 in 10,000). The risk at the NOEL/100 is estimated to be less than $10^{-4} \times$ NOEL/LED_{01}. There is a difference of opinion as to the NOEL from those given by Crump (*4*) for ethylenethiourea and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (intestinal anomalies), indicating one of the difficulties of basing procedures on the NOEL.

Results and Discussion

Comparison of the NOEL and the LED_{01} for the 10 data sets is shown in Table 2. The NOEL was a factor of 2 to 6 times the LED_{01}, resulting in estimated upper limits of risks at the NOEL/100 to be 2 to 6 times higher than the estimated upper risk of 10^{-4} at the $LED_{01}/100$. Due to the lack of detail of the published data, the issue of litter effects could not be addressed. Ignoring the differences among litters could result in a reduced estimate of the variance. Hence, the LED_{01} values reported are larger than would be obtained when the variation of litters is properly included. Thus, the NOELs for these 10 data sets are actually more than 2 to 6 times greater than the LED_{01} values that would be obtained if interlitter variation were included.

The approach presented here for extrapolating risk to low dose levels based upon the LED_{01} takes into account the dose-response data. Therefore, it provides a stronger basis on which to estimate risk for low dose levels. More research is needed for developing appropriate dose-

Table 1. Experimental proportions of affected animals (quantal data).

Compound	Species	Effect	Doses	Number
Ethylenethiourea (*11*)	Rats	Fetal anomalies	0 mg/kg	0/167
			5	0/132
			10[a]	1/138
			20	14/81
			40	142/178
			80	24/24
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (*12*)	Rats	Intestinal anomalies	0 μg/kg	0/24
			0.125	0/38
			0.250[a]	1/33
			0.500	3/31
			1.00	3/10
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (*13*)	Rats	Fetal mortality	0 μg/kg/day	22/318
			0.001[a]	16/224
			0.010	17/100
Calcium valproate (*14*)	Rabbits	Skeletal malformations	0 mg/kg	3/93
			50	7/136
			150[a]	2/95
			350	35/83
Ametantrone (*15*)	Rabbits	Malformations	0 mg/kg	3/92
			0.2[a]	2/55
			0.4	6/66
			0.8	8/68
Linamarin (*16*)	Hamsters	Skeletal defects	0 mg/kg	1/67
			70[a]	0/55
			100	5/56
			120	10/54
			140	14/63
Solanidine (*17*)	Hamsters	Resorbed fetuses	< 1 mg/kg	90/1054
			22	26/312
			66[a]	49/464
			110	70/235
Paraxanthine (*18*)	Mice	Limb anomalies	0 mg/kg	0/114
			175[a]	2/87
			300	32/91
		Palate anomalies	0	0/114
			175[a]	1/87
			300	30/91
		Eye anomalies	0	0/114
			175[a]	0/87
			300	9/91

[a]NOEL.

Table 2. Estimated upper limits on reproductive or developmental risk at the NOEL/100.

Chemical	Species	End point	$LED_{01}/100$[a]	NOEL/100	Estimated risk at NOEL/100
Ethylenethiourea	Rat	Anomalies	0.062	0.100	$< 1.6 \times 10^{-4}$
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Rat	Intestinal	0.0004	0.0025	$< 6.2 \times 10^{-4}$
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Rat	Mortality	5.4×10^{-6}	10.0×10^{-6}	$< 1.9 \times 10^{-4}$
Valproate	Rabbit	Skeletal	0.41	1.50	$< 3.7 \times 10^{-4}$
Ametantrone	Rabbit	Malformations	0.0005	0.0020	$< 4.0 \times 10^{-4}$
Linamarin	Hamster	Skeletal	0.20	0.70	$< 3.5 \times 10^{-4}$
Solanidine	Hamster	Resorptions	0.25	0.66	$< 2.6 \times 10^{-4}$
Paraxanthine	Mouse	Limb	0.29	1.75	$< 6.0 \times 10^{-4}$
		Palate	0.32	1.75	$< 5.5 \times 10^{-4}$
		Eye	0.46	1.75	$< 3.8 \times 10^{-4}$

[a]Estimated risk at the $LED_{01}/100$ is 10^{-4}.

response models for these types of data. Improved statistical curve-fitting procedures of experimental data can provide better estimates of the LED_{01}. For the reproductive and developmental effects examined here, the use of a 100-fold safety factor applied to the NOEL resulted in estimated risks of less than 6×10^{-4}. The risk of reproductive or developmental effects is estimated to be less than 10^{-4} at the $LED_{01}/100$ in the animal test

systems. Thus, a lower level of risk can generally be obtained by using the $LED_{01}/100$ rather than the NOEL/100 to set allowable dose levels. If lower levels of potential risk are desired, then larger safety factors must be used.

REFERENCES

1. Lehman, A. J., and Fitzhugh, O. G. 100-fold margin of safety. Bull. Assoc. Food Drug Offic. 18: 33–35 (1954).
2. Jackson, B. A. Safety assessment of drug residues. J. Am. Vet. Med. Assoc. 176: 1141–1144 (1980).
3. Gaylor, D. W. The use of safety factors for controlling risk. J. Toxicol. Environ. Health 11: 329–336 (1983).
4. Crump, K. S. A new method for determining allowable daily intakes. Fundam. Appl. Toxicol. 4: 854–871 (1984).
5. Dourson, M. L., Hertzberg, R. C., Hartung, R., and Blackburn, K. Novel methods for the estimation of acceptable daily intake. Toxicol. Ind. Health 4: 23–33 (1985).
6. Mantel, N., and Bryan, W. R. Safety testing of carcinogenic agents. J. Natl. Cancer Inst. 27: 455–470 (1961).
7. Van Ryzin, J. Quantitative risk assessment. J. Occup. Med. 22: 321–326 (1980).
8. Farmer, J. H., Kodell, R. L., and Gaylor, D. W. Estimation and extrapolation of tumor probabilities from a mouse bioassay with survival/sacrifice components. Risk Anal. 2: 27–34 (1982).
9. Kimmel, C. A., and Gaylor, D. W. Issues in qualitative and quantitative risk analysis for developmental toxicology. Risk Anal. 8: 15–20 (1988).
10. Howe, R. B., and Crump, K. S. GLOBAL82: A computer program to extrapolate quantal animal toxicity data to low doses. K. S. Crump and Company, Inc., Ruston, LA 1982.
11. Khera, K. S. Ethylenethiourea: Teratogenicity studies in rats and rabbits. Teratology 7: 243–252 (1973).
12. Khera, K. S., and Ruddick, J. A. Polycholorodibenzo-*p*-dioxins: Perinatal effects and the dominant lethal test in Wistar rats. In: Advances in Chemistry Series, No. 120, Chlorodioxins-Origins and Fate. American Chemical Society, 1973.
13. Murray, F. J., Smith, F. A., Nitschke, K. D., Humiston, C. G., Kociba, R. J., and Schwetz, B. A. Three generation reproduction study of rats given 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50: 241–252 (1979).
14. Petrere, J. A., Anderson, J. A., Sakowski, R., Fitzgerald, J. E., and de la Iglesia, F. A. Teratogenesis of calcium valproate in rabbits. Teratology 34: 263–269 (1986).
15. Petrere, J. A., Kim, S.-N., Anderson, J. A., Fitzgerald, J. E., de la Iglesia, F. A., and Schardein, J. L. Teratology studies of ametantrone acetate in rats and rabbits. Teratology 34: 271–278 (1986).
16. Frakes, R. A., Sharma, R. P., and Willhite, C. C. Developmental toxicity of the cyanogenic glycoside linamarin in the golden hamster. Teratology 31: 241–246 (1985).
17. Renwick, J. H., Claringbold, W. D. B., Earthy, M. E., Few, J. D., and McLean, A. C. S. Neural-tube defects produced in Syrian hamsters by potato glycoalkaloids. Teratology 30: 371–381 (1984).
18. York, R. G., Randall, J. L., and Scott, W. J., Jr. Teratogenicity of paraxanthine (1,7-dimethylxanthine) in C57BL/6J mice. Teratology 34: 279–282 (1986).